



doi: <https://doi.org/10.20546/ijcrar.2020.808.004>

Review on Phosphate Solubilizing Fungi and Its Inoculation to Seeds

Chala Dandessa*

Department of Biology, Jimma Teachers College, P. O. Box 95, Ethiopia

*Corresponding author

Abstract

Phosphorous is one of the most vital nutrients for plant development. It increases the strength of cereal straw in plants, stimulates flower formation and fruit production, as well as stimulates root development and it's needed for seed development. Owing to their high retention in soil, movement of phosphate ions in the soil is very little. In this regard, the supply of P through biological systems is considered a practicable substitute, and inoculation of Phosphate solubilizing microorganisms, particularly fungi to soil, is a consistent source to increase soluble P in soil. Though phosphorus is found to be a restraining factor in many soils, application of PSMs as biofertilizers or bioconverters for solubilizing fixed phosphorus has not been practiced effectively. Many fungal species can solubilize rock phosphate, aluminum phosphate and tricalcium phosphate. Yeast belonging to genus *Saccharomyces*, *Hansenula*, *Klockera*, *Rhodotorula* and *Debaryomyces* spp. were phosphate solubilizing yeasts. For application of phosphate solubilizing fungi to seeds, the surface of seeds should be sterilized, cleaned with sterile water and dried out. The surface disinfected seeds can be coated by way of soaking seeds in liquid culture medium of phosphate solubilizing fungi for 2 hr; while inoculation of the seed using plant and both *Aspergillus niger* + *Penicillium notatum* is recommended. Consequently, the application of P solubilizing fungi is commended as a workable way to increase crop yield.

Article Info

Accepted: 08 July 2020

Available Online: 20 August 2020

Keywords

Biofertilizers, Fungi, Phosphate solubilizing fungi, Seed inoculation.

Introduction

One of the vital nutrients that affect a plant development and metabolic processes is phosphorus. In cell energy production; based on the availability of phosphorous to form Adenosine Triphosphates (ATP), phosphorus remarkably influences nucleic acid synthesis, sugar and energy production, photosynthesis, and also stimulates N₂ fixation in legumes. Also, it supports root development, seed and flower formation, maturity and production, stem and stalk strength, quality of crop and opposition to plant diseases. Owing to their great retention in soil, movement of phosphate ions is very slow in the soil. Plants' retrieval degree of P fertilizer is

merely around 10 to 30% (Birhanu *et al.*, 2017a). Phosphorous is connected with the fusion of a chain of essential cellular molecules, for example nucleic acids, phospholipids, nucleotides, etc. Dissolvable biochemical fertilizers are rapidly restrained in the soil, so it is unavailable for plants intake. Approximately 70 to 90% of phosphorus fertilizer applied to the soil is precipitated by Ca, Fe and Al metal cations making insoluble forms which are not efficiently taken up by plants (Walpolo and Yoon, 2012). Consequently, they have to develop alternate and cleaner agricultural methods. On this subject, P supply by means of biological systems is treated as a practical substitute, as well as injection of Phosphate solubilizing microorganisms to soil,

particularly fungi, is a trustworthy source to upsurge soluble P in soil (Microorganisms and Online, 2018). Phosphate-solubilizing fungi have been recounted in various environmental places, for example arctic areas, volcanic areas, forest, agricultural fields, mine areas, vermicompost, mangrove, etc. Resulting from injection, phospho-fungi have been seen to increase growth of various plants such as fibre and oilseed crops, vegetables, horticultural crop, cereals, and legumes, etc. Generally, the use of bacterial inoculants, mostly the phospho-fungi, as an alternative for artificial phosphatic fertilizers has been considered valid to increase plant development and economical to maintain the natural veracity and productiveness of soil (S. and J. Malviya, 2018).

Phosphate solubilizing fungi

Phosphate solubilizing fungi (PSF) are fungi that offer a biologically tolerable mean to convert insoluble phosphate to soluble forms, making them accessible for plant to absorb. Thus far, numerous fungal strains (*Penicillium* and *Aspergillus*) and bacterial strains (*Bacillus*, *Rhizobium*, *Enterobacter* and *Pseudomonas*) have been known to be potent phosphate solubilizers. Through the process of chelation, exchange reactions acidification, and production of organic acid, insoluble phosphates are adapted into accessible forms using phosphate solubilizing microorganisms. Though phosphorus is seen as a restrictive factor in numerous soils, applying PSMs as bioconverters or biofertilizers to solubilize stable phosphorus has not been effectively practiced so far (Walpola and Yoon, 2012).

Consistent with some studies, injection of phosphate solubilizing *Bacillus species* as well as *Pseudomonas* has led to greater phosphorus uptake and more grain harvest of wheat (*Triticum aestivum L.*) afterwards. Single and double inoculations with phosphorus fertilizer led to about 30-40% rise in grain produce of wheat equated to phosphorus fertilizer only and double inoculation without phosphorus fertilizer enhanced grain produce up to 20% compared to sole phosphorus fertilization. *Pseudomonas* inoculation had advantageous consequence on salt lenience of *Zea mays L.* under NaCl stress (Walpola and Yoon, 2012).

Just about 70 to 90% of phosphorus fertilizer added to the soil is triggered by Ca, Fe and Al metal cations creating forms that are insoluble that plants cannot competently consume. Injection of phosphate solubilizing microbes in the soil is proven to increase

solubilization of insoluble phosphates leading to higher crop performances (Walpola and Yoon, 2012). For successful cultivation of plants, nutrient management is a vital factor. Biofertilizers can have impact on crop's quantity and quality. Low phosphate solubility is a vital cause of plant development limitation in several soils. Numerous microbes improve phosphate solubility, but a minute part is well-known on the degree of their phosphorus solubilizing capability. A study on the native populations of phosphate-solubilizing fungi and bacteria was carried out on diverse rhizospheres soil models gotten from banana plant and its outcome on spinach crop (*Amaranthus cruentus L.*) so as to match the outcomes. Some study concentrates on the phosphate-solubilizing ability of fungi and bacteria in rhizospheres soil models gotten from banana plant, showing the domination of *Aspergillus species* (234.12 mm) as major phosphate solubilizers, with *Bacillus subtilis* (160.82 mm), afterward *Pseudomonas aeruginosa* 126.11, *Penicillium sp.*, 99.02 and *Micrococcus sp.*, 89.4. Potent solubilizers were identified as *A. niger* and *B. subtilis* in some studies. Therefore, effort was put in place to enhance the phosphate solubilization of possible solubilizers at diverse pH with temperature. It was established that fungi and bacteria similarly exhibited extreme phosphate solubilization at pH 3.0 with its precise temperature at 28⁰ C and 37⁰ C (Reena *et al.*, 2013).

Phosphate solubilizing species of fungi

A different collection of fungi and bacteria is known to be associated with microbial phosphate solubilization mechanisms through which insoluble forms of phosphates are changed to soluble forms (HPO_4^{-2} or H_2PO_4^-). The main procedures accredited to the alteration are exchange reactions, chelation, acidification of the medium and production of various acids (Walpola and Yoon, 2012).

It was reported that numerous fungi are capable of solubilizing phosphate only with ammonium as the nitrogen source. The source of nitrogen in salt form appears to be significant, since it was essential to increase phosphate solubilization of rock phosphate. Earlier reports on phosphorus solubilizing microorganisms have credited the dissimilarities in phosphate solubilization (while nitrate and ammonium were used) to the use of diverse mechanisms for the production of acidity in the culture (Pradhan and Sukla, 2005a).

From such examinations, different kinds of phosphate solubilizing microbes have been effectively recognized. Numerous fungal species can solubilize rock phosphate, aluminum phosphate and tricalcium phosphate, such as *Aspergillus awamori*, *Aspergillus tubingensis*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Penicillium italicum*, *Penicillium rugulosum*, *Penicillium radicum*, *Fusarium oxysporum*, *Humicola sp.*, *Curvularia lunata*, *Aerothecium sp.*, *Pythium sp.*, *Sclerotium rolfsii*, *Phoma sp.*, *Cladosporium sp.*, *Rhizoctonia sp.*, *Cunninghamella spp.*, *Rhizoctonia solani*, *Rhodotorula sp.*, *Oideodendron sp.*, *Candida sp.*, *Schwanniomyces occidentalis*, *Pseudonymnoascus sp.* The soil yeasts *Candida tropicalis*, *Geotrichum capitatum*, *Geotrichum candidum*, *Rhodotorula rubra* and *Rhodotorula minuta* solubilized insoluble phosphate (Birhanu *et al.*, 2017b). Yeast belonging to genus *Saccharomyces*, *Hansenula*, *Klockera*, *Rhodotorula* and *Debaryomyces spp.* were phosphate solubilizing yeasts (Gizaw *et al.*, 2017).

Phosphorus is plentiful in soils, in inorganic and organic forms equally; however, it is inaccessible to plants. Consequently, soil becomes phosphorus (P)-deficient, making P one of the most vital nutrient elements that limit crop efficiency. To avoid the P deficiency, phosphate-solubilizing microorganisms plays a significant role in making P accessible for plants by melting insoluble phosphorous. Fungi display qualities such as biological control, mineral solubilization, and making of secondary metabolites (Khan *et al.*, 2010).

Characterization of phosphate solubilizing fungi

So as to isolate the PSF, it can be cultured on Pikovskaya's (PVK) medium after collection of PSF samples from the soil. After culture prepared on PVK media there will be formation of hallow zone from where PSF solubilize the phosphorous. The groups enclosed with a halo zone can be moved to PVK medium to preserve the culture's purity (Reena *et al.*, 2013). Phosphorus solubilizing microorganisms are stated to liquefy insoluble phosphates by producing organic or inorganic acids and/or through the reduction of the pH (Pradhan and Sukla, 2005a). Most preceding reports state that calcium phosphates are liquefied by acidification (Pradhan and Sukla, 2005a). According to Elias *et al.*, phosphate solubilization on PVK medium can be observed through the growth of diverse isolates on PVK medium. After injection, PVK medium can be incubated at 28 °C for fungi, for about 5 days. The solubility of phosphate was examined as a zone of authorization with

a diameter measured in millimeters and taken in triplicate. The microbial phosphorus solubilization trait was examined through defining the P-solubilization efficiency (PSE). $PSE = \text{diameter of entire colony} / \text{diameter of clearing zone} \times 100$. The effective P-solubilizing bacterial species were then further recognized (Reena *et al.*, 2013). Phosphate solubilization index will be determined through the use of the ensuing formula: ratio of the total diameter (colony + halo zone) and the colony diameter (Elias *et al.*, 2016)

Solubilization Index (SI)

$$= \frac{\text{colony diameter} + \text{halo zone diameter}}{\text{colony diameter}}$$

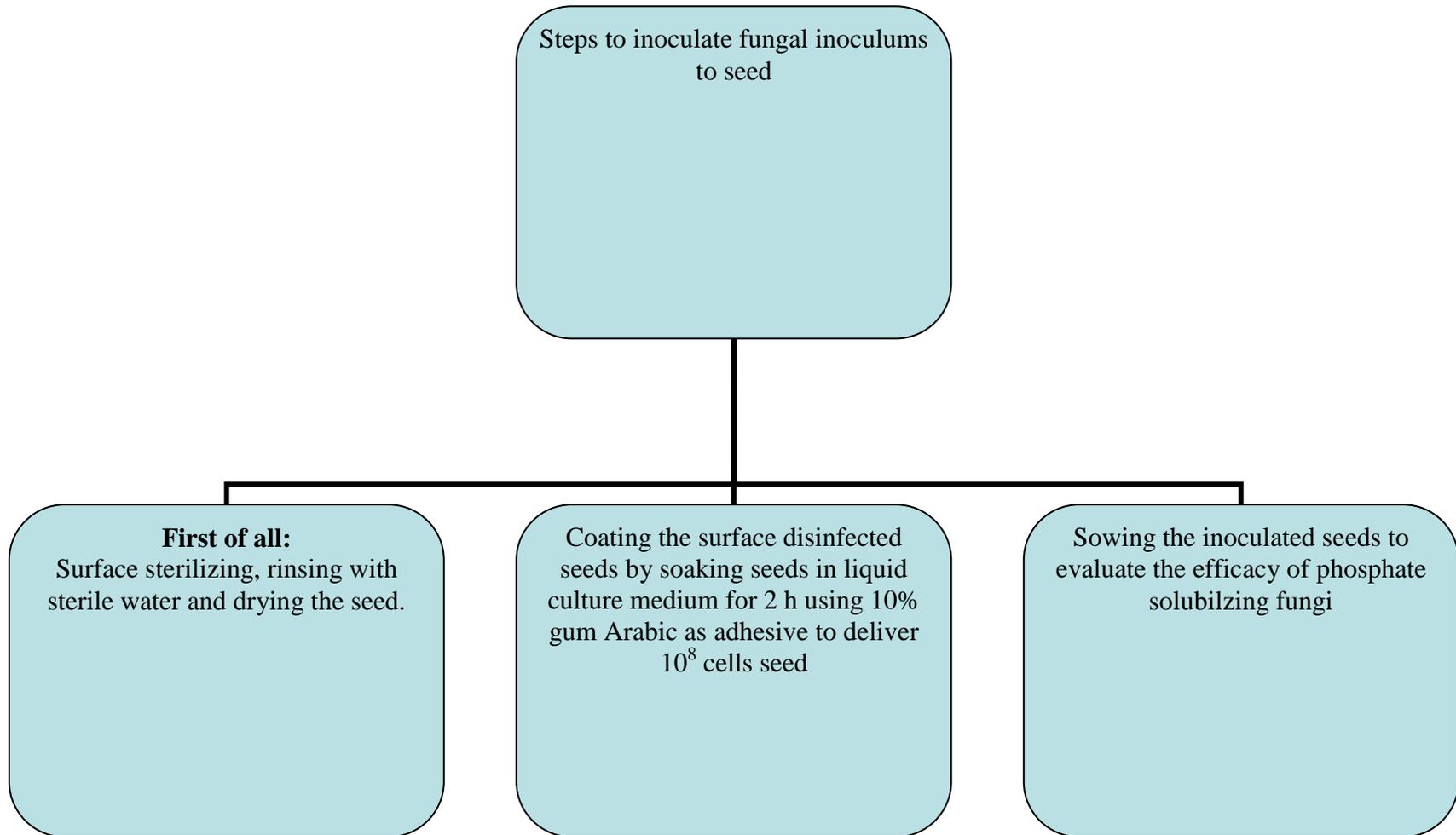
In order to identify the isolated fungus strain for a correct species, morphological characterization, 18S rRNA gene sequencing and phylogenetic analysis of the isolated fungus can be used. The morphology characteristics of the isolate can be evaluated according to the methods described by (Wu Yingben *et al.*, 2012). Additionally it can be characterized by performing physiological and biochemical as per standard procedures by plate assays (Wu Yingben, He Yuelin, Yin Hongmei, Chen Wei, Wang Zhen, 2012).

Sources of phosphate solubilizing fungi

The soil is the habitation of a massive, compound and collaborative community of natural soil organisms; whose actions chiefly control the physico-chemical properties of the soil and subsequently encourage the development of the plants. From seed germination to maturation of a plant, such plant lives in close relationship with organisms in the soil (Mohammad *et al.*, 2007).

Fungi are the vital mechanisms of soil microbes naturally establishing more of the soil biomass than bacteria; dependent on depth of the soil and nutrient circumstances. It has been reported that fungi have better capability to solubilize insoluble phosphate than bacteria. A wide-ranging soil fungi are stated to solubilize insoluble phosphorous such as *Penicillium sp.* and *Aspergillus niger*, which are the most common fungi proficient for of phosphate solubilization (Gizaw *et al.*, 2017).

Figure.1 In a general for application of phosphate solubilizing fungi to seeds the following brief steps will be recommended



Inoculation of phosphate solubilizing fungi to seeds

Most agricultural soils encompass huge reserves of phosphorus (P); a significant quantity is amassed due to consistent use of P fertilizers. Conversely, a larger part of soil phosphorus, about 95–99%, is present in the form of insoluble phosphates and thus cannot be used by the plants. Therefore, inoculating fungal strains that have the potential to solubilize insoluble inorganic phosphates has a vital role. Fungal species such as *Penicillium sp.* and *Aspergillus sp.* are known for their prospect to solubilize insoluble phosphate amassed in the soil. Generally, Phosphate solubilizing microorganisms transform insoluble phosphates into soluble forms through the processes of chelation, exchange reactions and acidification. Therefore such microorganisms possibly will not only recompense for higher cost to manufacture fertilizers in industries but to also activate fertilizers added into the soil (Pradhan and Sukla, 2005b) (Fig. 1).

For application of phosphate solubilizing fungi to seeds, the seeds should be surface sterilized, rinsed with sterilized water and dried up. The surface sterilized seeds can be covered by wringing seeds in liquid culture medium of phosphate solubilizing fungi for 2 hr. While inoculation of the seed using plant and both *Aspergillus niger* + *Penicillium notatum* is recommended. Consequently the use of P solubilizing fungi is suggested as a workable way to increase crop yield (J. Malviya, Singh, and Joshi, 2011).

In conclusion, phosphate-solubilizing fungi are an important contributor in microbial P-mobilization and would be important possible way to increase available P for plant. Phosphate solubilizing fungi (PSF) are fungi that offer a biologically tolerable mean to convert insoluble phosphate to soluble forms, making them accessible for plant to absorb. Accordingly, from the present review we can conclude that the improvement of soil with the application of P-solubilizing fungi is suggesting as a sustainable way for increasing crop yield and also improve the physico-chemical properties of the soil. It is recommended to use combination of different species of phosphate solubilizing microorganisms.

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How to cite this article:

Chala Dandessa. 2020. Review on Phosphate Solubilizing Fungi and Its Inoculation to Seeds. *Int.J.Curr.Res.Aca.Rev.* 8(8), 25-30. doi: <https://doi.org/10.20546/ijcrar.2020.808.004>